

Pyrrolizidine Alkaloids from *Lithospermum canescens* Lehm.

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Seven pyrrolizidine alkaloids (PAs) have been isolated from *Lithospermum canescens* and their structures determined by spectroscopical methods. Besides the known lycopsamine, *O*⁷-acetyl-lycopsamine and *O*⁷-acetylintermedine four new PAs were found. Their structures are *O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(+)-trachelanthoyl-heliotridine (= *O*⁷-(3-hydroxy-3-methyl-butanoyl)-rinderine = canescine), *O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(-)-viridifloryl-heliotridine (= *O*⁷-(3-hydroxy-3-methyl-butanoyl)-echinatine = canescenine) and their *O*¹³-acetyl-derivatives (= acetylcanescine; acetylcanescenine).

Key words: *Lithospermum canescens*, Pyrrolizidine Alkaloids, Canescine and Derivatives

Introduction

Lithospermum canescens Lehm., Boraginaceae (native American name: “hoary puccoon”) grows in open prairies in northern USA and southern Canada. Because it contains pigments of the shikonin-type (Wiedenfeld *et al.*, 1998) it is used as a body dye by native people (Densmore, 1928).

As *L. canescens* belongs to the Boraginaceae family the presence of pyrrolizidine alkaloids (PAs) could be expected.

Based on structural aspects (double-bond in position 1,2; esterification at both necic OH-functions) PAs can show toxic effects. Toxicity occurs not only after oral administration but also after percutaneous absorption although to a smaller extent than by ingestion (Brauchli *et al.*, 1982). Thus, according to the German Federal Health Bureau regulations the sale of PA containing products for external use is restricted in Germany to a daily dose of 100 µg and a maximum use of six weeks per year (Bekanntmachung, 1992). Aerial parts of *L. canescens* were therefore investigated. Seven PAs were isolated and their structures determined by GC-mass spectroscopy and homo- as well as heteronuclear 2D-NMR correlated spectroscopy. Four of them have not been described previously. The known alkaloids belong to the retronecine-type and are *O*⁹-(-)-viridifloryl-retronecine (= lycopsamine), its *O*⁷-acetyl-derivative (= acetyllycopsamine)

and *O*⁷-acetyl-*O*⁹-(+)-trachelanthoyl-retronecine (= acetylintermedine). The new PA show the structures of *O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(+)-trachelanthoyl-heliotridine (= *O*⁷-(3-hydroxy-3-methyl-butanoyl)-rinderine), *O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(-)-viridifloryl-heliotridine (= *O*⁷-(3-hydroxy-3-methyl-butanoyl)-echinatine), *O*¹³-acetyl-*O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(+)-trachelanthoyl-heliotridine, *O*¹³-acetyl-*O*⁷-(3-hydroxy-3-methyl-butanoyl)-*O*⁹-(-)-viridifloryl-heliotridine.

Based on structure-toxicity relationships (Wiedenfeld and Roeder, 1984) toxic side effects must be expected for all substances found.

Results and Discussion

Aerial parts of *L. canescens* were extracted as previously described (Roeder and Wiedenfeld, 1977; Wiedenfeld and Roeder, 1979). From the crude alkaloidal extract **1–7** were isolated (Fig. 1).

The GC-MS spectrum of **1** shows the [M]⁺-peak at 299 indicating the formula C₁₅H₂₅NO₅. Those of **2** and **3** show [M]⁺-Peaks at 341 corresponding to the formulas C₁₇H₂₇NO₆. The further MS fragmentation and also the NMR data of **1–3** are as described earlier (Wiedenfeld and Roeder, 1991; Roeder *et al.*, 1982; Kelley and Seiber, 1992; Roitman, 1983).

The [M]⁺-Peak in the GC-MS spectrum of **4** and **6** occurs at 399 indicating the molecular formulas

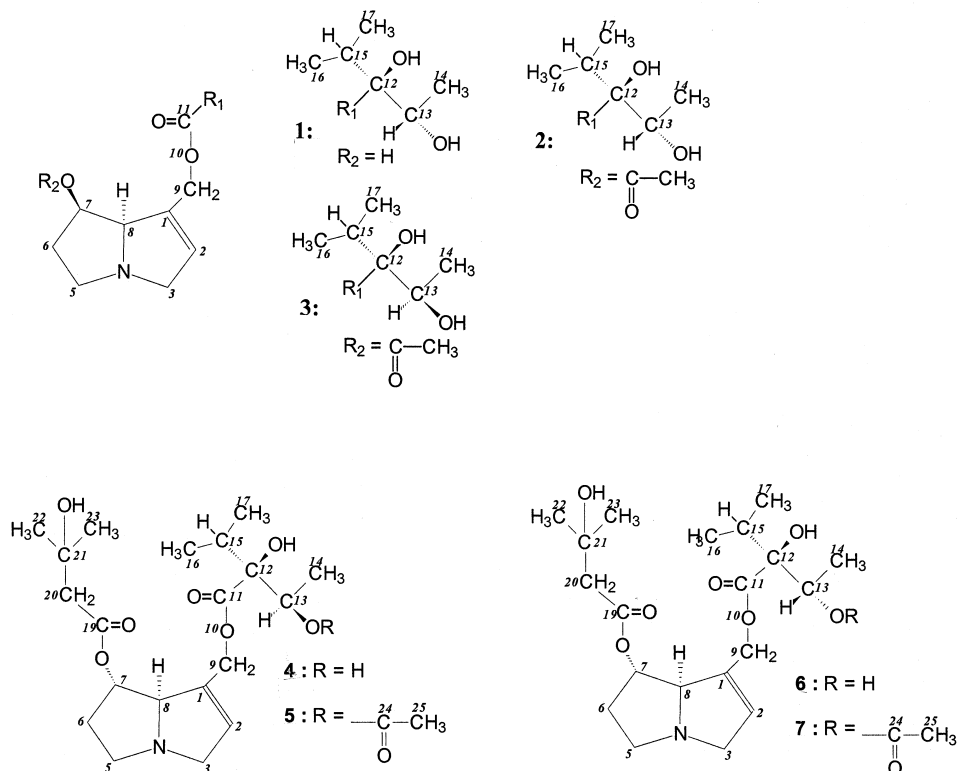


Fig. 1: Structures of the isolated Pas: Lycopsamine (**1**), O⁷-acetyl-lycopsamine (**2**), O⁷-acetylintermediate (**3**), O⁷-(3-hydroxy-3-methyl-butanoyl)-O⁹-(+)-trachelanthoyl-heliotridine = canescine (**4**), O⁷-(3-hydroxy-3-methyl-butanoyl)-O⁹-(-)-viridifloryl-heliotridine = canescenine (**5**), O⁷-acetylcanescine (**6**), O⁷-acetylcanescenine (**7**).

C₂₀H₃₃NO₇. Loss of CH₃ leads to *m/z* 384. The ions *m/z* 355 and 338 result from [M]⁺-C₂H₄O and further loss of OH. The cleavage of the ester function at O-9 leads to *m/z* 256 and *m/z* 238. The decay of the O⁷-acid is demonstrated by an ion at *m/z* 220 (loss of OH at C-21), *m/z* 180 (loss of C₃H₇O) and cleavage of the ester function to *m/z* 136. The fragments *m/z* 136, 120, 93 and 80 are typical for retronecine or its isomer heliotridine. The ms spectra of **5** and **7** show the [M]⁺-Peaks at 441 corresponding to the formulas C₂₂H₃₅NO₈. After loss of an acetyl function (indicated by *m/z* 441–426–398) the further fragmentations are similar to those of **4** and **6**; they differ only in intensities.

The ¹H- and ¹³C-NMR-data of **4–7** are summarized in the Experimental part. The assignment was performed by interpretation of H,H- and C,H-correlated spectra. Important structural information is provided by the ¹³C chemical shifts of C-6 (~ 34 ppm), C-7 (~ 74 ppm) and C-8 (~ 75 ppm)

(Jones *et al.*, 1982; Mohanraj and Herz, 1982; Wiedenfeld and Roeder, 1991). These signals establish **4–7** as diesters of heliotridine. This is further confirmed by the ¹H and ¹³C shift for C-9: ~ 4,7/62 ppm as well as by the ¹H data for C-7: ~ 5.4 ppm. The esterifying acid at O-7 is identical for all 4 PA and is characterised by the values for the methylene group C-20 (2.5 and 47 ppm), C-21 (69 ppm) and the methyl groups 22/23 (1.3 and ~ 29 ppm). These data proof the structure of a 3-hydroxy-3-methyl-butanoyl ester. The NMR data for the O-9 acid in **6** are the same as in **1** leading to the structure of a (-)-viridiflorylester. **4** shows differences in the data for H-13 (4.07 instead of 3.89 ppm) and for C-16 and C-17 (17.8 and 14.2 instead of 17.2 and 17.1 ppm). The stereochemistry at C-12 can be deduced by interpretation of the shift difference of the C-9H₂ AB-system (Mohanraj and Herz, 1982; Wiedenfeld and Roeder, 1991). For this aspect values from 0–0.2 ppm indicate an

S-configuration while higher values indicate *R*-configuration. The configuration at C-13 is shown by the H-13 and C-13 data as well as by the shift differences of the C-NMR data for the methyl groups C-16/C-17 (Wiedenfeld and Roeder, 1991). Thus, the values for C-13 (4.07 and 69.5 ppm instead of 3.89 and 71.1 ppm) and C-16/C-17 ($\Delta\text{Hz} = 3.6$ instead of 0.1 ppm) give evidence for a C-12*S* and a 13*R* configuration (= (+)-trachelanthic acid). The data for **5** and **7** differ from those in **4** and **6** only in a downfield shifting of H-13 and C-13 (~ 5.2 and ~ 74 ppm) and the additional data for an acetyl group (~ 2.0 and ~ 21 ppm for CH_3 and 170 ppm for $\text{C}=\text{O}$) which confirm the structures as O-13-acetyl-(+)-trachelanthic and a O-13-acetyl(-)-viridifloric acid, respectively. These data proof the structures of *O*⁷-(3-hydroxy-3-methylbutanoyl)-*O*⁹-(+)-trachelanthoyl-heliotridine (= *O*⁷-(3-hydroxy-3-methylbutanoyl)-rinderine) (**4**), *O*⁷-(3-hydroxy-3-methylbutanoyl)-*O*⁹-(-)-viridifloryl-heliotridine (= *O*⁷-(3-hydroxy-3-methylbutanoyl)-echinatine) (**6**), *O*¹³-acetyl-*O*⁷-(3-hydroxy-3-methylbutanoyl)-*O*⁹-(+)-trachelanthoyl-heliotridine (**5**), *O*¹³-acetyl-*O*⁷-(3-hydroxy-3-methylbutanoyl)-*O*⁹-(-)-viridifloryl-heliotridine (**7**).

For the new PAs we propose the names canescine (**4**), canescenine (**6**), acetylcanescine (**5**) and acetylcanescenine (**7**).

All isolated PAs are expected to produce toxic side effects. The PA content (GC) was about 0.02% (dry weight). Therefore, based on the German regulations for external use of preparations from PAs containing plants, application of more than 0.5 g dried plant material per day may expose the user to a health risk.

Experimental

General

NMR-spectra (Bruker AC 400) were measured in $\text{CDCl}_3/\text{D}_6\text{-DMSO}$ at 400 and 100 MHz, respectively. GC-MS: GC: 150° (5 min.) – 250 °C, 10°/min; HP-1, 25 m \times 0.32 mm; Inj.: 250 °C, det.: 280 °C; **1**: 12.67 min, **2**: 13.49 min, **3**: 13.88 min, **4**: 16.79 min, **5**: 17.51 min, **6**: 16.92 min, **7**: 18.14 min; MS: 220 °C; interface: 250 °C; 2000 emV.

Plant material

Plants were collected in July 2000 at Parkland Bot, Togo, Saskatchewan, Canada. A voucher specimen is deposited at the Department of Biology and Pharmaceutical Botany, Medical University of Warsaw, Poland.

Extraction and isolation

Extn. of plant material (aerial parts; 500 g) was carried out as described earlier (Roeder and Wiedenfeld, 1977; Wiedenfeld and Roeder, 1979). Prep. TLC [silica gel F_{254} , $\text{CH}_2\text{Cl}_2\text{-MeOH-NH}_4\text{OH}$ (25%), 75:24:1 v/v/v] yielded the alkaloids as oils (6 mg **1**, 5 mg of **2** and **3**, 8 mg **5** and **7**, 10 mg **4**, 1 mg **6**).

Canescine (**4**)

GC-MS *m/z* (%): $[\text{M}]^+$ $\text{C}_{20}\text{H}_{33}\text{NO}_7$ 399 (0.41); $\text{C}_{19}\text{H}_{30}\text{NO}_7$ 384 (4.10); $\text{C}_{18}\text{H}_{28}\text{NO}_6$ 355 (0.82); $\text{C}_{18}\text{H}_{27}\text{NO}_5$ 338 (0.25); $\text{C}_{13}\text{H}_{20}\text{NO}_4$ 256 (9.84); $\text{C}_{13}\text{H}_{20}\text{NO}_3$ 238 (66.0); $\text{C}_{13}\text{H}_{18}\text{NO}_2$ 220 (21.4); $\text{C}_{10}\text{H}_{14}\text{NO}_2$ 180 (11.6); $\text{C}_8\text{H}_{10}\text{NO}$ 136 (41.6); $\text{C}_8\text{H}_{10}\text{N}$ 120 (100); $\text{C}_6\text{H}_7\text{N}$ 93 (64.7); $\text{C}_5\text{H}_6\text{N}$ 80 (20.1). ^1H NMR: δ (ppm): 5.83 (d, $J_{2,3a} = 1.6$ Hz, 1H, H-2), 5.38 (ddd, $J_{7,8} = 1.9$ Hz, $J_{7,6} = 1.8$; 1.6 Hz, 1H, H-7), 4.79 (dd, $J_{9a,9b} = 13.9$ Hz, $J_{9a,8} = 9.1$ Hz, 1H, H-9A), 4.67 (dd, $J_{9b,9a} = 13.9$ Hz, $J_{9,8} = 6.2$ Hz, 1H, H-9B), 4.35 (m, 1H, H-8), 4.07 (q, $J_{13,14} = 6.4$ Hz, 1H, H-13), 3.95 (ddd, $J_{3a,3b} = 11.4$ Hz, $J_{3a,8} = 3.2$ Hz, $J_{3a,2} = 1.6$ Hz, 1H, H-3A), 3.38 (dddd, $J_{3b,3a} = 11.4$, $J_{3b,8} = 3.2$, $J_{3b,5a} = 2.0$ Hz, $J_{3b,2} = 1.6$ Hz, 1H, H-3B), 3.33 (dm, $J_{5a,5b} = 10.7$, 1H, H-5A), 2.98 (3OH), 2.65 (ddd, $J_{5b,5a} = 10.7$, $J_{5b,6} = 7.8$, $J_{5b,7} = 1.8$ Hz, 1H, H-5B), 2.45 (s, 2H, $\text{H}_2\text{-20}$), 2.09 (dm, $J_{6,5b} = 10.7$, 2H, $\text{H}_2\text{-6}$), 2.00 (qq, $J_{15,16/17} = 6.8$ Hz, 1H, H-15), 1.26 (s, 6H, $\text{H}_3\text{-22/23}$), 1.19 (d, $J_{14,13} = 6.4$ Hz, 3H, $\text{H}_3\text{-14}$), 0.94 (d, $J_{16,15} = 6.8$ Hz, 3H, $\text{H}_3\text{-16}$), 0.91 (d, $J_{17,15} = 6.8$ Hz, 3H, $\text{H}_3\text{-17}$), ^{13}C NMR: δ (ppm): 175.1 (C-11), 171.7 (C-19), 133.0 (C-1), 127.5 (C-2), 83.4 (C-12), 75.4 (C-8), 74.4 (C-7), 69.5 (C-13), 69.1 (C-21), 62.6 (C-3), 62.3 (C-9), 53.6 (C-5), 46.9 (C-20), 34.4 (C-6), 33.1 (C-15), 29.4 (C-22), 29.2 (C-23), 17.2 (C-14), 17.2 (C-16), 17.1 (C-17).

Canescenine (**6**)

GC-MS *m/z* (%): $[\text{M}]^+$ $\text{C}_{20}\text{H}_{33}\text{NO}_7$ 399 (0.10); $\text{C}_{19}\text{H}_{30}\text{NO}_7$ 384 (1.56); $\text{C}_{18}\text{H}_{28}\text{NO}_6$ 355 (0.41);

C₁₈H₂₇NO₅ 338 (0.26); C₁₃H₂₀NO₄ 256 (9.59); C₁₃H₂₀NO₃ 238 (67.5); C₁₃H₁₈NO₂ 220 (20.6); C₁₀H₁₄NO₂ 180 (14.7); C₈H₁₀NO 136 (45.1); C₈H₁₀N 120 (100); C₆H₇N 93 (61.6); C₅H₆N 80 (20.2). ¹H NMR: δ (ppm): 4.74 (dd, *J*_{9a,9b} = 12.9 Hz, *J*_{9a,8} = 6.2 Hz, 1H, H-9A), 4.73 (dd, *J*_{9b,9a} = 12.9 Hz, *J*_{9,8} = 6.4 Hz, 1H, H-9B), 3.89 (q, *J*_{13,14} = 6.6 Hz, 1H, H-13), ¹³C NMR: δ (ppm): 71.1 (C-13), 16.0 (C-14), 17.8 (C-16), 14.2 (C-17). Further data are similar to **4**.

Acetylcanescine (**5**)

GC-MS *m/z* (%): [M]⁺ C₂₂H₃₅NO₈ 441 (0.73); C₂₁H₃₂NO₈ 426 (2.60); C₁₈H₂₈NO₆ 355 (2.36); C₁₃H₁₉NO₄ 255 (6.26); C₁₃H₂₀NO₃ 238 (62.4); C₁₃H₁₈NO₂ 220 (18.3); C₁₀H₁₄NO₂ 180 (38.9); C₈H₁₀NO 136 (47.2); C₈H₁₀N 120 (100); C₆H₇N 93 (73.9); C₅H₆N 80 (19.9). ¹H NMR: δ (ppm): 5.21 (q, *J*_{13,14} = 6.4 Hz, 1H, H-13), 2.00 (s, 3H, H₃-25), ¹³C NMR: δ (ppm): 170.4 (C-24), 72.4 (C-13), 21.2 (C-25). Further data are similar to **4**.

Acetylcanescenine (**7**)

GC-MS *m/z* (%): [M]⁺ C₂₂H₃₅NO₈ 441 (1.23); C₂₁H₃₂NO₈ 426 (2.38); C₂₀H₃₂NO₇ 398 (1.56); C₁₈H₂₈NO₆ 355 (1.39); C₁₃H₁₉NO₄ 255 (5.66); C₁₃H₂₀NO₃ 238 (58.2); C₁₃H₁₈NO₂ 220 (20.3); C₁₀H₁₄NO₂ 180 (44.3); C₈H₁₀NO 136 (43.4); C₈H₁₀N 120 (100); C₆H₇N 93 (80.3); C₅H₆N 80 (22.1). ¹H NMR: δ (ppm): 5.17 (q, *J*_{13,14} = 6.6 Hz, 1H, H-13), 2.03 (s, 3H, H₃-25), ¹³C NMR: δ (ppm): 170.4 (C-24), 73.7 (C-13), 21.3 (C-25). Further data are similar to **6**.

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